# BACTERIAL POLYSACCHARIDE

## RELATED APPLICATION

This is a division of application Ser. No. 889,163 filed Mar. 23, 1978, which is a continuation-in-part of application Ser. No. 842,646, filed Oct. 17, 1977, now abandoned.

## BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention pertains to a novel heteropolysaccharide which is produced by the action of a bacteria on a selected carbon source. Further, the invention pertains to a novel process for producing a heteropolysaccharide by bacterial fermentation of a selected carbon source under controlled conditions.

2. Description of the Prior Art

It is known that heteropolysaccharides can be produced by certain microorganisms. Some of such heteropolysaccharides function as hydrophilic colloids and because of their viscosity properties and rheology have been used as thickening agents for aqueous systems. Illustrative of prior art heteropolysaccharides, their preparation and uses are U.S. Pat. Nos. 3,020,207; 3,256,271; 3,894,976; 3,915,800 and 3,894,976.

As with other fields of technology, research has continued with the objective of discovering new heteropolysaccharides having useful properties as thickening, suspending and/or stabilizing agents.

## OBJECTS OF THE INVENTION

It is an object of this invention to provide a new heteropolysaccharide. It is another object to provide a method for making this new compound. A still further object is provision of formulations containing our new heteropolysaccharide as a thickening or suspending or stabilizing agent. These and other objects of the invention will be apparent from the following description of this invention.

## SUMMARY OF THE INVENTION

It has now been found that a high viscosity anionic heteropolysaccharide composed of about 33% mannose, 29% glucose, 21% galactose and about 17% glucuronic acid and also containing about 5.7% acetyl and about 4.9% pyruvate is obtained by an aerobic fermentation of an organism isolated from a soil sample from the Canal Zone. This heteropolysaccharide has desirable thickening, suspending and/or stabilizing properties in aqueous systems.

## DETAILED DESCRIPTION

The heteropolysaccharide of this invention is a high molecular weight polysaccharide containing primarily carbohydrate residues and a minor amount of protein. It is sometimes referred to as a "gum" but it is believed that the heteropolysaccharide terminology is more accurate and precise. In the following description of our invention it will sometimes be referred to as Heteropolysaccharide S-21.

The bacterium employed in the process of the present invention which is identified as Strain tTR-45, is a mutant of *Klebsiella pneumoniae* Strain S-21 that was isolated from the rhizosphere soil of a plant of the genus *Aechmea epiphytes* belonging to the pineapple family. The soil sample was obtained in the Canal Zone. Strain tTR-45 requires thymine for growth at 37° C. but does not require thymine for growth at 30° C. A deposit of Strain tTR-45 was made with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, on 11 August 1977 under Accession No. ATCC 31314. The culture is available to the public without restriction.

This organism requires a fermentation medium that supplies a carbon source, a phosphorus source, a nitrogen source, a magnesium source and an iron source. The carbon source typically is hydrolyzed starch with a DE range of 12–31. The starch can be hydrolyzed with commercially available α-amylases. The phosphorus source may be either $Na_2HPO_4$, $NaH_2PO_4$, $K_2HPO_4$ or $KH_2PO_4$ or a mixture thereof. The concentration may range from about 0.025 to about 0.5%. The magnesium source may be supplied with $MgCl_2$ or $MgSO_4$ in concentrations of from about 0.005 to about 0.02%. The nitrogen source may be $NaNO_3$, $KNO_3$, $NH_4NO_3(NH_4)_2SO_4$, or $NH_4Cl$ as well as organic sources such as soy peptone Type T (Sheffield Chemical, Norwich, New York), Promosoy 100 (Central Soya Chemurgy Division), NZ-amine Type A (Sheffield), or Ferm Amine Type IV (Sheffield). The medium may contain either inorganic or organic nitrogen or mixtures thereof. The concentration of inorganic nitrogen in the medium may range from about 0.045 to about 0.2% and with the organic nitrogen from about 0.01 to about 0.1%. The iron may be supplied to the fermentation as $FeCl_3$ or $FeSO_4$ at levels of 1–10 ppm.

The pH of this fermentation preferably is maintained between about 6.3 and about 7.7, and the temperature between about 28° C. and about 33° C. for maximum polysaccharide production.

The fermentation time is typically from about 48 to 60 hours when proper conditions of medium, temperature, pH and other fermentation parameters are met.

## DETAILS OF PROCEDURES

The soil sample when received is plated onto yeast-malt (YM) agar, E-1 agar with 1% dextrose and E-1 agar with 1% 42 dextrose equivalent (DE) corn syrup, and an isolate, Strain 21, is picked from a YM agar plate and pure cultured on nutrient agar.

Strain S-21 is plated on minimal medium containing thymine and trimethoprim and incubated at 37° C. for 4 days. Those colonies which grow on the plate are tested for a thymine requirement at 37° C. Those strains that required thymine at 37° C. are tested again for thymine requirement at 30° C. and 37° C. Strain tTR-45 is one of five strains that did not require thymine at 30° C. but did at 37° C. The reversion frequency on minimal medium is about $2\times10^{-8}$ revertants/cell.

This mutant can be cultured on blood agar, chocolate agar, brain heart infusion agar, and nutrient agar. These rich media all have sufficient thymine to meet the thymine requirement of tTR-45. This mutant does not grow on minimal medium at 37° C. but will grow on minimal medium with added thymine. The minimum thymine requirement is greater than 5 ppm but less than 10 ppm.

The minimal medium is prepared as follows:

| Salt Solution | |
|---|---|
| $K_2HPO_4$ | 10.5 gm. |
| $KH_2PO_4$ | 4.5 gm. |
| $(NH_4)_2SO_4$ | 1.0 gm. |
| Sodium citrate . $2H_2O$. | 0.5 gm. |